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Characterization of PLA-embedded mesenchymal cells in vitro and in an osteochondral repair model

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Purpose: To elucidate the survival of mesenchymal cells (MC) in the polylactic acid scaffold (PLA) in vitro and the possibility of MC/PLA constructs for osteochondral repair in vivo.
Methods and Materials: Bone marrow cells of mature male rabbits were cultured for 2 weeks, and fibroblast-like MC, which contain mesenchymal stem cells, were obtained. A biodegradable PLA core with 1 million MC was rotated at 100 rpm to create MC/PLA construct. After 1 week culturing, the construct was transplanted into an osteochondral defect in the medial femoral condyle of female rabbits. To examine the survivability of transplanted MC, the male-derived sex-determining region Y gene was detected in the defect as a MC marker by PCR technique.
Results: Approximately 93% of the original 1×10^6 MC attached to the PLA within 2 hours and increased up to 9.8×10^5 in a week in vitro. The cartilaginous matrix was recognized by safranin O staining, however the PLA matrix was still present in the defects at 24 weeks after transplantation in vivo. During the time passage, transplanted MC number decreased from 7.8×10^5 at 1 week, 3.5×10^5 at 4 weeks, to 3.8×10^4 at 12 weeks. Finally, transplanted cells were unable to be detected at 24 weeks.
Conclusions: MC increased in number in vitro, and could contribute to the osteochondral repair with expressing the cartilaginous matrix, however the number was decreasing with time (i.e. 24 weeks) in vivo. These results could be essential for achieving cartilage regeneration by cell transplantation strategies with growth factors and/or gene therapies.

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NMDA receptor signalling in human osteoarthritic chondrocytes.

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Purpose: Human OA chondrocytes are known to express several subunits of the NMDA receptor (NMDAR), a neuronal signalling molecule. This study analyses downstream activation events and interactions of NMDAR with molecules known to be involved in chondrocyte mechanotransduction.
Methods and Materials: OA chondrocytes human knee joint arthroplasty specimens were used. Cell membrane potentials of cells were measured following stimulation with NMDA (50mM, 10 min) or mechanical stimulation (MS, 0.33Hz, 20 min) in the +/- either EGTA. Nifedipine gadolinium, apamin, tetrodotoxin, NR2B9c-TAT (disrupts NMDAR-PSD95 association), anti-IL1 receptor, anti-IL4 receptor, and anti–integrin antibodies.
Results: NMDA stimulation of OA chondrocytes results in a membrane depolarisation response that is inhibited by removal of extracellular calcium by EGTA. Nifedipine, gadolinium, apamin and anti-IL4 had no effect on membrane depolarisation whilst treatment with NR2B9c-TAT completely inhibited membrane depolarisation. Whereas in the presence of tetrodotoxin NMDA and MS resulted in membrane hyperpolarisation response in OA chondrocytes; no change in membrane potential was seen when cells were stimulated in the presence of both apamin and tetrodotoxin. Anti-IL1R and b1-integrin antibodies were shown to block NMDA induced membrane depolarisation.
Conclusions: Activation of OA chondrocyte NMDAR results in a signal cascade that requires extracellular calcium and association of NMDAR with PSD95. The membrane depolarisation results from activation of a tetrodotoxin sensitive sodium channel. Blockade of the NMDA induced-depolarisation by anti-IL1R and anti-b1integrin antibodies suggests activation of an autocrine/paracrine loop and association of NMDAR with integrins. MS of OA chondrocytes results in b1-integrin and IL1b dependent membrane depolarisation and NMDAR maybe involved in this mechanotransduction process.

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An analysis of chondral and sub-chondral cell viability in constructs devoid of soft tissue attachement.

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Purpose: The purpose of this study was to evaluate cell viability in bone and cartilage constructs devoid of soft tissue attachment.
Methods and Materials: Methods: A total of 24 rabbit anterior femoral condyle constructs were harvested from living specimens under sterile conditions. The condyles were transferred to the sub-fasical plane in the midline thoacodorsal area. The rabbits were segregated into three groups of eight. The first group of eight had their construct harvested at two weeks, the second group at three weeks, the third group at four weeks. The articular cartilage and bone was then examined histologically for intact nuclear material to determine cellular viability
Results: The 24 constructs demonstrated similar findings. Reactive fibrosis surrounded islands of bone with a cap of articular cartilage. The cartilage was viable with no evidence of chondrocyte cell death. The underlying subchondral bone was viable for over 2mm. Metaphyseal and morrow elements demonstated early necrosis.
Conclusions: Previous examination of articular cartilage has demonstrated that it relies predominantly on anaerobic metabolism. The bone beneath the cartilage appears to have retained the same anaerobic capability. The inflammatory environment in high energy wounds may actually facilitate the perfusion and continued viability of cartilage and subchondral osteocytes. Preservation of osteoarticular constructs after trauma may be warranted. Future designing of peri-articular reconstruction equipment and the use of bone morphogenic proteins in acute fractures are concepts worth considering.

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An evaluation of articular cartilage and subchondral bone architecture (Articulone) in bovine cadaver knees after high energy trauma

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Purpose: There is relatively little information about the injury pattern or the perfusion of subchondral bone after high energy trauma to the darthrodial joint. The purpose of this study was to evaluate the fracture pattern at the metaphyseal and subchondral bone interface after exposure to high energy blast effect.
Methods and Materials: Methods: Twelve adult bovine knees from the hindquarters of twelve different animals were harvested with the capsule left intact. High energy blast waves were directed at ten of the knees from a sagital direction. Two of the knees were exposed to a high energy blast wave from a coronal vector.
Results: Grossly twelve of the specimens manifested diaphyseal bone disruption. The blast wave severely damaged the diaphyseal portion of both the tibia and the femur leaving the joint somewhat intact. Joint penetration and involvement ranged between 15 to 60 percent of the total joint surface for both the femur and the tibia. In all areas tested there was a distinct anatomic bony disruption at the metaphyseal and subchondral bone junction. This was demonstrated via visula and radiographic examination of the specimens.
Conclusions: High energy trauma to bovine specimen knees appears to validate clinical observations that there is a shearing phenomenon at the subchondral bone and metaphyseal bone interface. Different regions of the extremity differed profoundly in their response to high energy trauma effect. The diaphysis appears to have been the most sensitive portion of the extremity to high energy trauma. Visual inspection as well as radiographic evaluation revealed that the joint surface generally fissured along the subchondral and metaphyseal bone interface even in the more profoundly affected portions of the joint. Similar phenomena has been noted in human victims of blast phenomena. Understanding of this phenomena may facilitate periarticular reconstruction after high energy trauma.